UTILITY PATENT APPLICATION TRANSMITTAL

Submit an original and a duplicate for fee processing

(Only for new nonprovisional applications under 37 CFR 1.53(b))

ADDRESS TO:

Assistant Commissioner for Patents
Box Patent Application
Washington, D.C. 20231

Attorney Docket No.	99,723
First Named Inventor	Shieh, et al
Express Mail No.	EL028729797US
Total Pages	32

APPLICATION ELEMENTS

1. ☒ Transmittal Form with Fee
2. ☒ Specification (including claims and abstract) [Total Pages 15]
3. ☒ Drawings [Total Sheets 4]
4. ☒ Oath or Declaration [Total Pages 3]
   a. ☒ Newly executed
   b. ☐ Copy from prior application **[Note Boxes 5 and 17 below]**
      i. ☐ Deletion of Inventor(s) Signed statement attached deleting inventor(s) named in the prior application
5. ☐ Incorporation by Reference: The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.
6. ☐ Microfiche Computer Program
7. ☐ Nucleotide and/or Amino Acid Sequence Submission
   a. ☐ Computer Readable Copy
   b. ☐ Paper Copy
   c. ☐ Statement verifying above copies

ACCOMPANYING APPLICATION PARTS

8. ☒ Assignment Papers
9. ☐ Power of Attorney
10. ☐ English Translation Document *(if applicable)*
11. ☐ Information Disclosure Statement (IDS)
    ☐ PTO-1449 Form
    ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Return Receipt Postcard (Should be specifically itemized)
14. ☐ Small Entity Statement(s)
    ☐ Enclosed
    ☐ Statement filed in prior application; status still proper and desired
15. ☐ Certified Copy of Priority Document(s)
16. ☒ Other: Certificate of Mailing by Express Mail

17. **If a CONTINUING APPLICATION**, check appropriate box and supply the requisite information:
    ☐ Continuation ☐ Divisional ☐ Continuation-in-part of prior application Serial No. .

APPLICATION FEES

BASIC FEE				$760.00
CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE	
Total Claims	20	-20=		x $18.00	$
Independent Claims	2	- 3=		x $78.00	$
☐ Multiple Dependent Claims(s) if applicable				+$270.00	$
				Total of above calculations =	$
				Reduction by 50% for filing by small entity =	$( )
☒ Assignment fee if applicable				+ $40.00	$40.00
				TOTAL =	$800.00

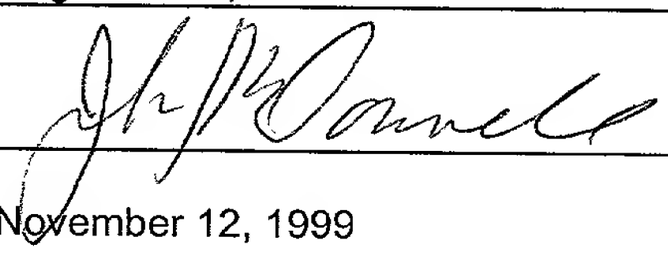
UTILITY PATENT APPLICATION TRANSMITTAL | Attorney Docket No. 99,723

18. ☐ Please charge my Deposit Account No. 13-2490 in the amount of $.

19. ☒ A check in the amount of $800.00 is enclosed.

20. The Commissioner is hereby authorized to credit overpayments or charge any additional fees of the following types to Deposit Account No. 13-2490:
    a. ☒ Fees required under 37 CFR 1.16.
    b. ☒ Fees required under 37 CFR 1.17.
    c. ☒ Fees required under 37 CFR 1.18.

21. ☒ The Commissioner is hereby generally authorized under 37 CFR 1.136(a)(3) to treat any future reply in this or any related application filed pursuant to 37 CFR 1.53 requiring an extension of time as incorporating a request therefor, and the Commissioner is hereby specifically authorized to charge Deposit Account No. 13-2490 for any fee that may be due in connection with such a request for an extension of time.

22. CORRESPONDENCE ADDRESS	
Name	**McDonnell Boehnen Hulbert & Berghoff**
Address	32nd Floor, 300 South Wacker Drive
City, State, Zip	Chicago, Illinois 60606
23. SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED	
Name	John J. McDonnell Reg. No. 26,949
Signature	
Date	November 12, 1999

UTILITY (Rev. 11/18/97)

A

CERTIFICATE OF MAILING BY "EXPRESS MAIL"
(NEW PATENT APPLICATION)

Express Mail No. EL028729797US

Deposited: November 12, 1999

I hereby certify that the attached correspondence, identified below, is being deposited with the United States Postal Service as "Express Mail Post Office to Addressee" under 37 CFR § 1.10 on the date indicated above and is addressed to the Asst. Commissioner for Patents, Box New Application, Washington, DC 20231.

By: [signature]
(person *actually* depositing)

Patent Application of: Chan-Long Shieh, Barbara Foley, Huinan Yu & Vi-En Choong

Title: Biochannel Assay for Hybridization with Biomaterial

X Patent Application (15 pages, including claims)

X Drawings (4 sheets)

X Postcard

X Transmittal Letter

X Assignment

X Oath & Declaration

X Check in the amount of $800.00

Attorney Docket No.:99,723

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(CASE No. 99, 723)
LS99-0042

TITLE: Biochannel Assay for Hybridization with biomaterial

INVENTORS: Chan-Long Shieh
6739 East Bar Z Lane
Paradise Valley, Arizona 85253,
A Citizen of USA

Barbara Foley
14842 South Foxtail Lane
Phoenix, AZ 85048,
A Citizen of USA

Huinan Yu
5760 W. Park Avenue
Chandler, AZ 85226,
A Citizen of Peoples Republic of China

Vi-En Choong
3380 W. Genoa Way
Chandler, AZ 85226,
A Citizen of Malaysia

ASSIGNED TO: MOTOROLA, INC.
1303 E. Algonquin Road
Schaumburg, IL 60196

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

The invention pertains to the structure, fabrication of a microfluidic device and methods for conducting analysis in microfluidic devices.

BACKGROUND OF THE ART RELATED TO THE INVENTION

Analysis utilizing specific binding pairs such as antigen/antibody; complementary DNA/DNA; DNA/RNA; RNA/RNA; biotin/avidin containing pairs are widely known in the art. Techniques for manufacturing and utilizing microfluidic devices are also well known. The art also discloses various techniques for DNA sequencing based on complementary binding of DNA.

DNA probe array technology, which utilizes binding of target single stranded DNA onto immobilized DNA probes has wide applications. A large amount of research and development activities have been carried out with different technology emphasis. For example, same technologies are focused on probe placement by mechanical means. Other technologies are focused on in-situ probe synthesis that is advantageous in producing large arrays. Additionally, other technologies are focused on gel pad arrays using photopolymerizaion and piezoelectric liquid dispensing technologies.

A common challenge to all DNA hybridization technologies is the lack of control of stringency for each individual probe site. The DNA hybridization process occurs at

specific temperature and salinity conditions and varies with DNA sequences. For DNA probe arrays, since the DNA probe sequences are different, hybridization recognition is never perfect under a uniform stringency condition for the entire probe array. The problem is most obvious for short duplexes which often results in single base mismatches. One can minimize the effect of mismatched hybridization by using large probe site redundancy. Stringency control has been provided for each probe site by controlling the electrophoretic movement of oligonucleotides. To successfully implement this later scheme, a meticulously engineered permeation layer is required to prevent DNA molecules or labeling agents being damaged by direct electrolysis or by the product of the electolysis.

In addition, the current DNA array technologies have failed to provide an effective solution to maximize hybridization efficiency. For diagnostic assays, the target DNA molecules are often of minute quantities. The detection limit of the assay is determined by the sensitivity of the detection device, and also by the amount of target oligos bound to the probes during the course of hybridization. In a stationary hybridization chamber where active mixing is absent, the probability of a given target molecule hybridizes to its complementary strand on the surface is determined by diffusion rate and statistics. It takes up to tens of hours for hybridization to complete at low target concentration levels. To better utilize the target molecules and enhance the hybridization, flow through technology has been proposed where the probe arrays are placed perpendicular to the fluidic flow direction. Even with flow through technology,

only a portion of the target molecules can come in contact with any specific DNA probe site.

The present invention overcomes the above technical issues by sequentially placing the DNA probe sites in microfluidic channels such that the DNA probe can efficiently contact its binding partner.

U.S. Patent 5,147, 607 describes a variety of microassay devices which have microchannels in plastic materials with a reagent such as an antibody or DNA immobilized on the channel at different locations. Techniques for binding antibodies to the microchannel wall are described but techniques for binding DNA are not described. The binding of probes to the microchannel wall does not provide for optimum contact of probe and test sample. U.S. Patent 5,843,767 describes microfabricated flowthrough porous apparatus for discrete detection of binding reactions such as DNA/DNA. WO/98/43739 describes porous flow channels having reagents immobilized in the chamber.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a schematic top view of a fluid channel filled with porous gel and spotted DNA probes.

Figure 2 shows lithographically patterned gel pads inside a microfluidic channel.

Figure 3 shows microfluidic channels with molded plastic microstructures for DNA attachment.

Figure 4 shows a microfluidic channel packed with beads where distinct sections of beads have a specific binding agent such as DNA.

Figure 5 illustrates a simple initial flow being directed into numerous channels.

Figure 6 illustrates a circulating microfluidic channel device.

SUMMARY OF THE INVENTION

The invention comprises microfluidic devices comprising a section of solid material such as a chip with a microchannel with an inlet and exit port for flowing fluids through the channels. The microchannel has separated defined regions of specific binding pair member immobilized on porous polymer, microstructures molded in the microchannels or packed beads. These structures provide for optimum contact of the immobilized binding pair member and a binding pair member in fluid flowing through the mirochannel. The porous polymer beads or microstructure must provide for flow and not obstruct the channel. The microchannel is operatively associated with a detector and a fluid propelling component to flow liquids in the channel and may also have electrodes at the exit and entrance ports.

DNA/DNA; DNA/RNA, and RNA/RNA complementary binding pair are preferred. The microchannel is operatively associated with target DNA labeled with a fluorophore, an excitation source and a detector to detect emitted fluorescence from the

binding pairs. It is an object of the invention to provide a method for DNA or RNA sequencing by providing the above identified chip with DNA or RNA probes immobilized in the separated defined region to bind fluorescently labeled target DNA.

It is also an object of the present invention to provide a means determining genetic defects. The invention also provides a means for identifying pathogens through DNA analysis.

The microchannels may have a variety of configurations, feedback arms, valves, and vents to control fluid flow. There may be single or multiple channels. The invention provides for efficient contact between immobilized binding substances and binding partners in the fluid flowing through the channel. The invention provides for improved hybridization stringency control by flow modulation; shortened assay time by increasing the rate of hybridization with flow induced agitation and by bringing the target and probe into proximity within the microfluidic channel; and increased hybridization efficiency which improves sensitivity. In addition there is no interference through hydrolysis.

DETAILED DESCRIPTION OF THE INVENTION

The chips microfluidic channels of the present invention are channels generally less than 200 microns in plastic with molding or embossing technology. The channels need to be of the dimension to support pumping of the microfluidic system The microfluidic channel may have any shape, for example, it may be linear, serpentine, arc shaped and the like. The cross-sectional dimension of the channel may be square, rectagular, semicircular, etc. There may be multiple and interconnected microchannels with valves to provide for recirculation.

The section of solid material maybe chips made of glass, ceramic, metal, silicon or plastic. Chips are preferably fabricated from plastics such as expoxy resin, polyacrylic resins, polyester resins, polystyrene, polycarbonate, polyvinyl chloride and the like. Specific binding pairs are DNA/DNA or DNA/RNA complementary binding pairs.

Fluid propelling components such as pressurized gas, vacuum, electric field, magnetic field and cetrifugal force devices are operatively associated with the microchannel to move fluid through the microchannel. In addition, charged test samples may be altered by modulating the electric field against or in the direction of the flow or perpendicular to the flow. Thus, the rate of fluid flow in the microchannel can be altered to promote binding of binding pairs, for example, hybridization of DNA/DNA or DNA/RNA pairs. Also, operatively associated with the microchannel is a detector such as an optical, electrical or electrochemical detector.

Figure 1 illustrates a serpentine shaped microfluidic channel 1 filled with porous gel 2 with discrete separate regions 3 which have attached a member of a specific binding pair. Such as DNA. Sample flows into the microfluidic channel 4 and exits the channel at 5. In this approach, the channel is filled with porous gel material such as agarose or polyacrylamide. The pores of the gel are made large enough by using dilute gelling solutions to permit significant fluid flow through the gel members of specific binding pair is spotted onto the gels so that the probes are chemically attached.

Figure 2 illustrates a microfluidic channel 10 which has patterned gel pads 11 within the channel. The gel pads are formed by photopolymerization of acrylamide using lithographic techniques.

Figure 3 illustrates a microfluidic channel 15 where high surface area microstructures are molded into the channel. Figure 3a shows a series of columns 16 in a distinct region and Figure 3b shows a distinct region of domes 17 molded into channel 15 These microstructures are chemically modified and specific binding substances are attached.

Figure 4 illustrates a microfluidic channel 20 packed alternately with regions of plain beads 21 and beads 22 having a specific binding substance, such as DNA.

Figure 5 illustrates a microfluidic channel 25 which branches in multiple microfluidic channels 26 a, b, c etc each of which have a distinct region of a binding

substance 27 as described above. Through this embodiment, a sample can be studied in parallel to test its reactivity to the same or different specific binding substance.

Figure 6 illustrates a chip 30 with a recirculating microfluidic channel 34. The microfluidic channel has discrete areas with specific binding substances 32 as described above and a recirculating arm33 and a valve 34 for output after recirculation. In this embodiment the test sample is recirculated past the location of the binding partner. Thus, dilute samples or slow reacting samples can be respectively passed by the specific binding substance.

Microfabricated plastic capillary electrophoresis (CE) devices have been demonstrated in the art. Thermoplastic molded polymethylmethacrylate CE devices are described by R.M. McCormick, et al, "Microchannel electrophoretic separations of DNA in injection-molded plastic substrates," *Anal. Chem.*, vol. 69, pp. 2626, 1997. Eckstrom *et al* investigated elastomeric polymers such as PDMS, "PCT Appl. WO91/16966," 1991. More recently, others have published electrophoretic separation of DNA ladders in PDMS devices, for example, C.S. Effenhauser, et al , "Integrated Capillary Electrophoresis on Flexible Silicone Microdevices," *Anal. Chem.*, vol. 69, pp.3451, 1997. Mastrangelo, et al describes building micro CE devices based on parylene-polycarbonate substrates using a surface micromachining approach, "An Inexpensive Plastic Technology for Microfabricated Capillary Electroophoresis Chip" presented at Micro-TAS'98, Banff, 1998. Thus, techniques are available for fabricating microchannels. The invention involves fixing specific binding substances by way of

porous polymer, beads or structure in the microchannel to more efficiently promote binding.

These examples are intended to illustrate the present invention and not to limit it in spirit or scope.

What is claimed:

1. A microfluidic device comprising a section of solid material with a microchannel having an entrance and exit port for the transportation of fluids in and out of the microchannel wherein the microchannel contains spacially separated defined regions of specific binding pair member immobilized on a porous polymer, beads or on microstructures fabricated in the microchannel.

2. The microfluidic device of claim 1 wherein the device is fabricated from silicon, glass, silicon dioxide, plastic or ceramics.

3. The microfluidic device of claim 1 wherein the separated defined regions are porous polymer with specific binding pair member bound to the porous polymer.

4. The microfluidic device of claim 1 wherein the separated defined regions have beads with specific binding pair member bound to the bead.

5. The microfluidic device of claim 1 wherein the defined regions are with immobilized binding pair members are formed by introducing hydrogels in the microchannels.

6. The defined region of claim 5 wherein the binding pair members are selectively dispensed on spatially separated portions of hydrogel.

7. The defined region of claim 5 wherein the hydrogels in the microchannels are patterned by means including photolithography.

8. The microfluidic device of claim 1 wherein the separated defined regions have microstructures fabricated into the microchannel and the microstructures have specific binding pair member bound thereto.

9. The microfluidic device of claim 1 wherein the binding pair members from a group consisting of DNA, RNA, polypeptides, nucleic acids, and antibody/antigens.

10. The microfluidic device of claim 1 wherein the specific binding member is a DNA or RNA probe.

11. The microfluidic device of claim 1 wherein the specific binding member is DNA.

12. The microfluidic device of claim 1, further comprising a fluid propelling component that is operatively associated with the microchannels.

13. The fluid propelling component in claim 12 is a pressurized gas, vacuum, electrical field, magnetic field or centrifugal force.

14. The microfluidic device of claim 1, comprising a detector component that is operatively associated with the microchannels.

15. The microfluidic device of claim 14 wherein the detector is an optical, electrical or electrochemical detector.

16. A method of detecting a specific binding member in a test sample comprising:

a. providing the microfluidic device of claim 1;

b. flowing the test sample through the microchannel to form a binding pair;

c. detecting the binding pair.

17. The method of claim 16 wherein the flow of the test sample is recirculated in the microchannel.

18. The method of claim 16 wherein the flow rate of the test sample is adjusted by a fluid propelling component operatively associated with the microchannel.

19. The method of claim 16 wherein the migration speed of a charged sample is further modified through applying a modular electrical field in or against the direction of flow.

20. The method of claim 16 wherein the charged test sample is attracted or repelled at the spatially defined region by the application of electrical field in the direction perpendicular to the flow direction.

ABSTRACT OF THE DISCLOSURE

The invention relates to a microfluidic device with microchannels that have separated regions which have a member of a specific binding pair member such as DNA or RNA bound to porous polymer, beads or structures fabricated into the microchannel. The microchannels of the invention are fabricated from plastic and are operatively associated with a fluid propelling component and detector.

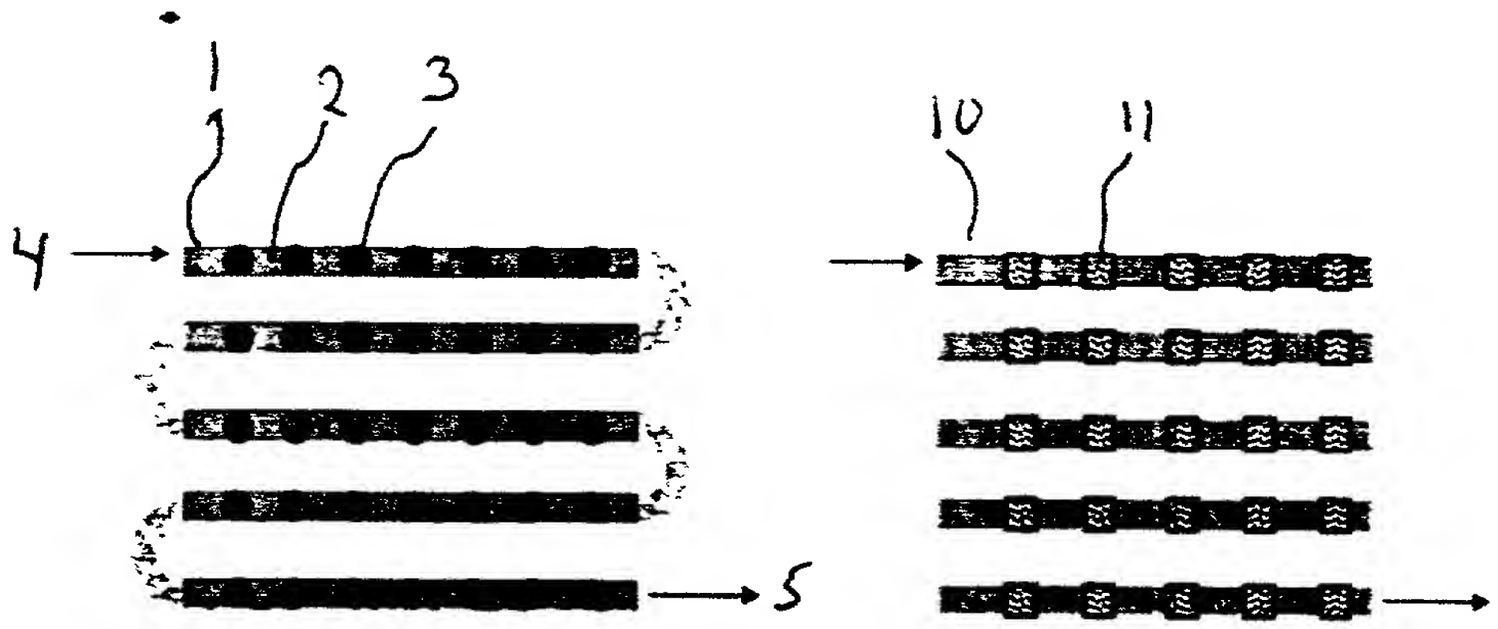


FIGURE 1

FIGURE 2

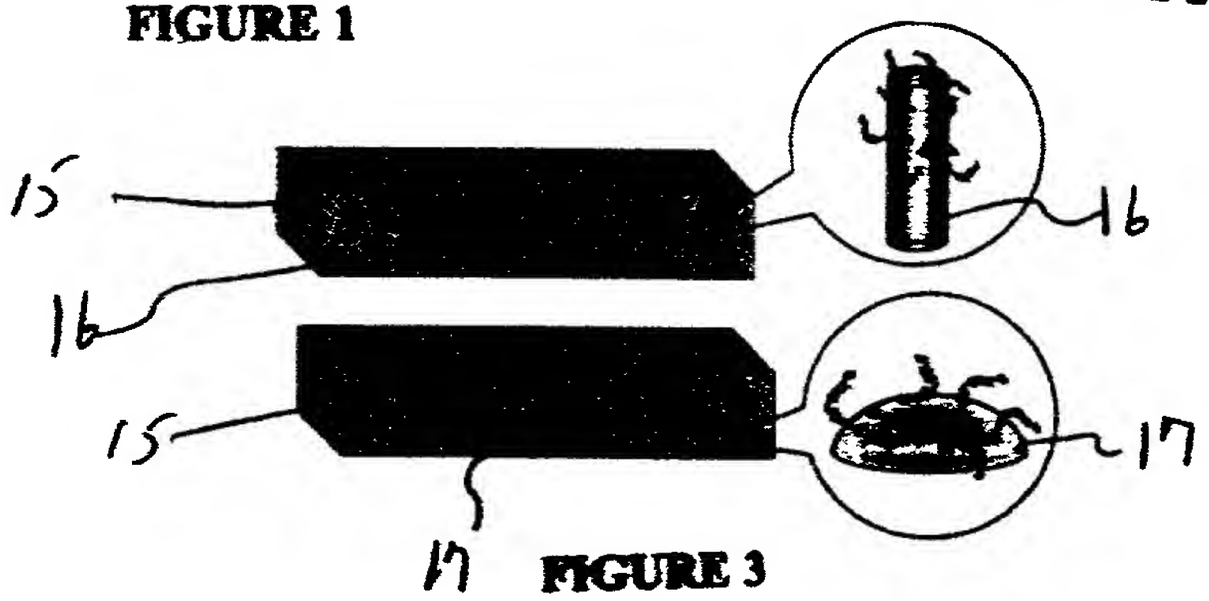


FIGURE 3

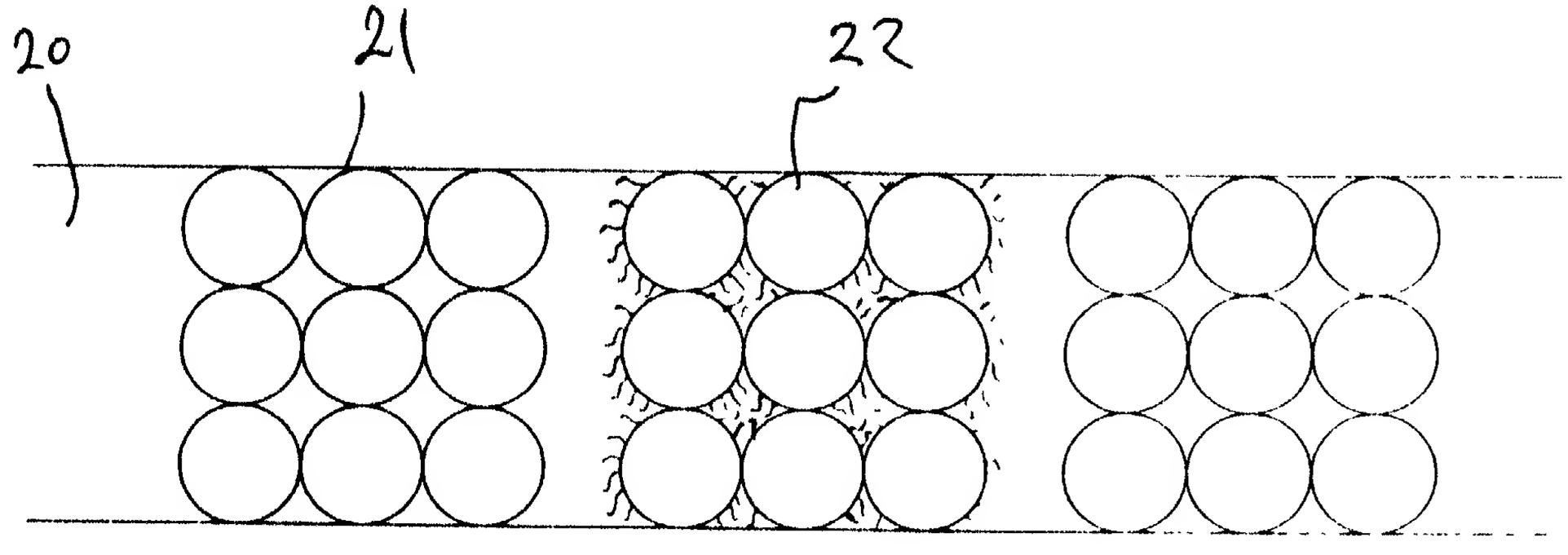


FIGURE 4

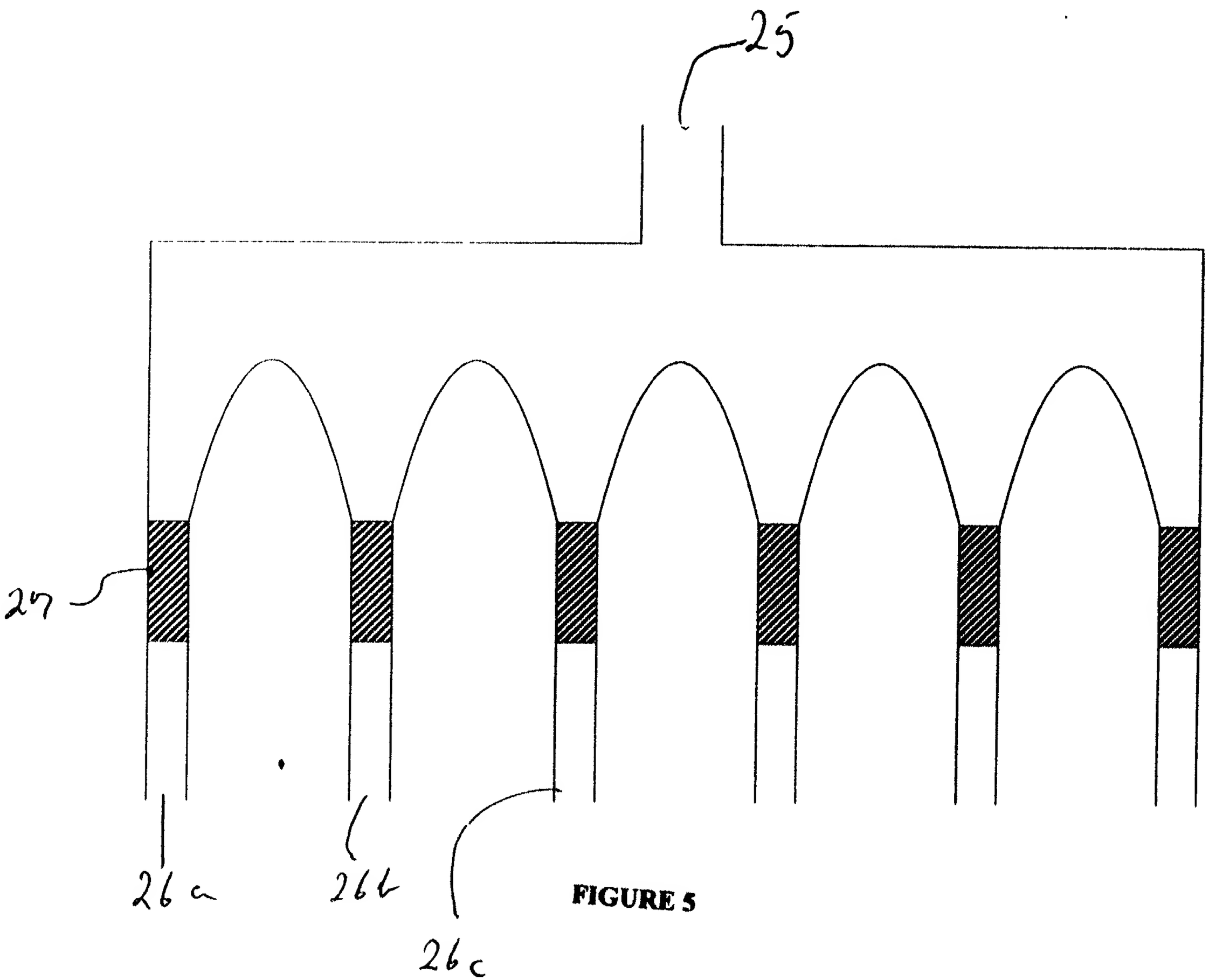


FIGURE 5

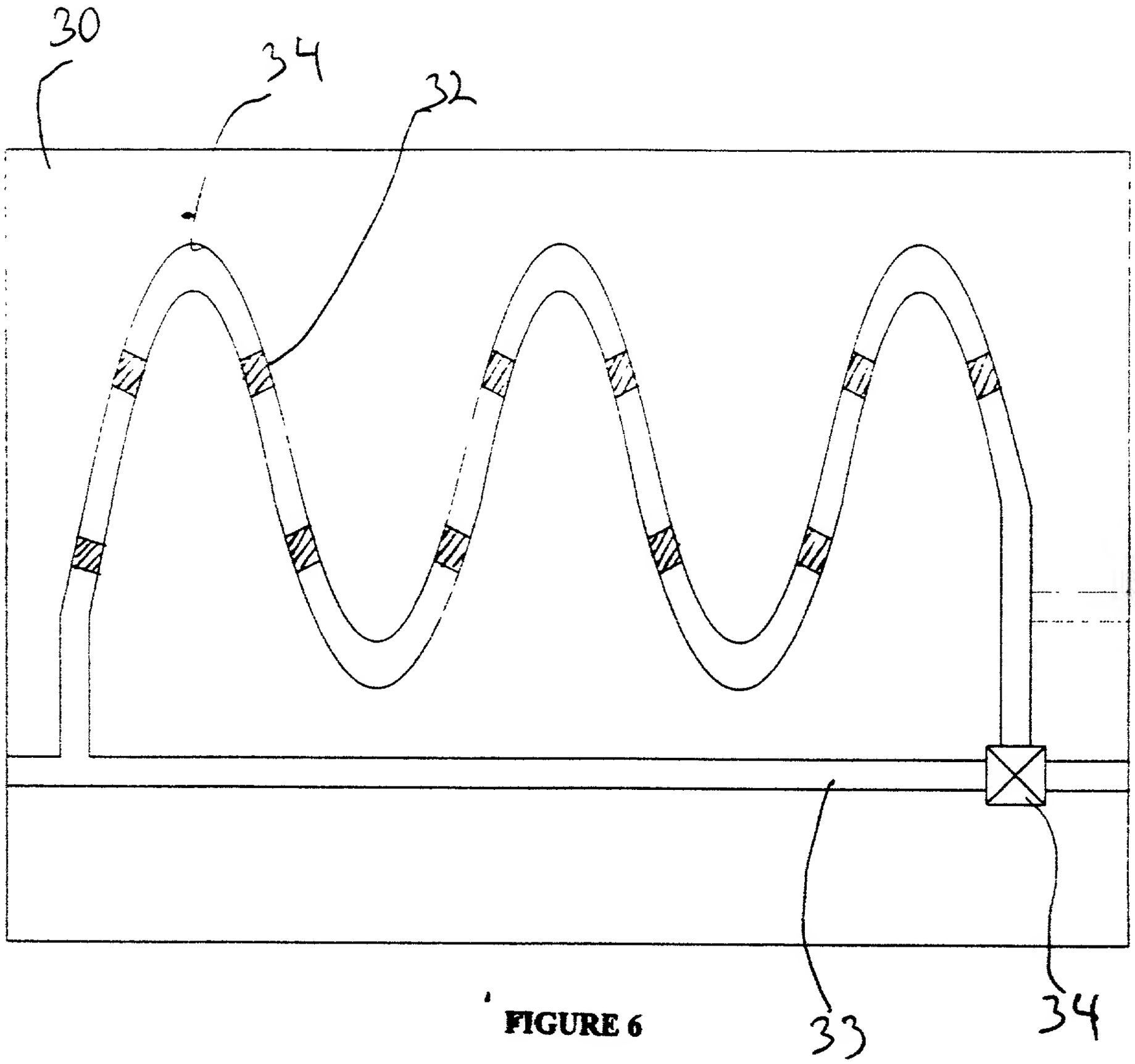


FIGURE 6

Case No.: 99,723

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

BIOCHANNEL ASSAY FOR HYBRIDIZATION WITH BIOMATERIAL

the specification of which is attached hereto unless the following space is checked:

☐ was filed on _____as United States Application Serial Number _____ _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

	Number	Country	Day/Month/Year Filed
1.			
2.			

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

	Application Number	Filing Date
1.		
2.		

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

	Application Number	Filing Date	Status: patented, pending, abandoned
1.			
2.			

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

I hereby appoint the following attorneys and agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

Denis A. Berntsen	Reg. No. 26707	Anthoula Pomrening (agent)	Reg. No. 38805
John J. McDonnell	Reg. No. 26949	George I. Lee	Reg. No. 39269
Daniel A. Boehnen	Reg. No. 28399	James M McCarthy	Reg. No. 39296
Bradley J. Hulbert	Reg. No. 30130	Jeremy Noe (agent)	Reg. No. 40104
Paul H. Berghoff	Reg. No. 30243	Sean M. Sullivan	Reg. No. 40191
Grantland G. Drutchas	Reg. No. 32565	Amir N. Penn	Reg. No. 40767
Steven J. Sarussi	Reg. No. 32784	Patrick J. Halloran (agent)	Reg. No. 41053
David M. Frischkorn	Reg. No. 32833	Joshua R. Rich	Reg. No. 41269
James C. Gumina	Reg. No. 32898	Thomas E. Wettermann	Reg. No. 41523
A. Blair Hughes	Reg. No. 32901	Vernon W. Francissen	Reg. No. 41762
Thomas A. Fairhall	Reg. No. 34591	Robert J. Irvine	Reg. No. 41865
Emily Miao	Reg. No. 35285	Richard A. Machonkin	Reg. No. 41962
Kevin E. Noonan	Reg. No. 35303	David S. Harper	Reg. No. 42636
Leif R. Sigmond, Jr.	Reg. No. 35680	Stephen Lesavich	Reg. No. 43749
Lawrence H. Aaronson	Reg. No. 35818	Enrique Perez	Reg. No. 43853
Matthew J. Sampson	Reg. No. 35999	Marcus J. Thymian	Reg. No. 43954
Curt J. Whitenack	Reg. No. 36054	S. Richard Carden (agent)	Reg. No. 44588
Christopher M. Cavan	Reg. No. 36475	Mark Chael (agent)	Reg. No. 44601
Michael S. Greenfield	Reg. No. 37142	Stephen H. Docter	Reg. No. 44659
Roger P. Zimmerman	Reg. No. 38670		

Address all telephone calls to John J. McDonnell at (312) 913-2110.
Address all correspondence to McDONNELL BOEHNEN HULBERT & BERGHOFF, 300 South Wacker Drive, Chicago, Illinois 60606 USA.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first inventor: Chan-Long Shieh

Inventor's signature: Chan-Long Shieh Date: 11/3/99
Residence: 6739 East Bar Z Lane, Paradise Valley, AZ
Citizenship: USA
Post Office Address: 6739 East Bar Z Lane, Paradise Valley, AZ

Full name of second joint inventor: Barbara Foley

Inventor's signature: Barbara Foley Date: 11/3/99
Residence: 14842 South Foxtail Lane, Phoenix, AZ
Citizenship: USA
Post Office Address: 14842 South Foxtail Lane, Phoenix, AZ

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001

Full name of third joint inventor: Huinan Yu

Inventor's signature: _____ Date: 11/3/99
Residence: 5760 W. Park Avenue, Chandler, AZ
Citizenship: Peoples Republic of China
Post Office Address: 5760 W. Park Avenue, Chandler, AZ

Full name of fourth joint inventor: Vi-En Choong

Inventor's signature: _____ Date: 11/3/99
Residence: 3380 W. Genoa Way, Chandler, AZ
Citizenship: Malaysia
Post Office Address: 3380 W. Genoa Way, Chandler, AZ

McDONNELL BOEHNEN
HULBERT & BERGHOFF
300 SOUTH WACKER DRIVE
CHICAGO, ILLINOIS 60606
TELEPHONE (312) 913-0001